Mutational Landscape and Actionable Biomarkers In Non-Small Cell Lung Carcinoma: A Next Generation Sequencing Based Study In Indian Cohort

SHORT SYNOPSIS

Submitted in partial fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

to

Manav Rachna International Institute of Research and Studies
(Deemed to be University)

by

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Certificate

I hereby declare that the short synopsis "**Mutational Landscape and Actionable Biomarkers In Non-Small Cell Lung Carcinoma: A Next Generation Sequencing Based Study In Indian Cohort"** being submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy Department of Faculty of Biotechnology under School of Engineering and Technology of Manav Rachna International Institute of Research & Studies (MRIIRS), Faridabad during the Academic Year – 2025-26.

It is a bona fide record of original work carried out under the supervision of Dr Kanchan Bhardwaj, Professor, School of Biotechnology and has not been submitted in full or part to any other university or institute for degree or diploma. Further, it is certified that short synopsis is plagiarism free and proof has been submitted.

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ABSTRACT

Immunotherapy, either alone or in combination with chemotherapy or targeted therapy, is now the standard of care for advanced Non-Small Cell Lung Cancer (NSCLC). The 5-year progression-free survival rate is low, which is 7.5-10%. Therefore, there is an urgent need to optimize treatment strategies for improvising clinical outcomes. The National Comprehensive Cancer Network (NCCN) has advised molecular testing, which involves using a comprehensive gene panel (CGP) testing. Each phenotype exhibits a distinct genotype. Earlier studies on CGP using NGS have revealed crucial signaling pathways and resistance mechanisms across multiple genes in each patient's tumor. The co-relation with PD-L1 expression has further guided the decision making towards patients' eligibility for Immunotherapy or combination therapies. The mutational landscape and resistance mechanisms of the Indian population remain less studied. Hence, this study aims to understand the mutational landscape, prevalence of resistance mechanisms and correlation of CGP with PD-L1 expression, in Indian population. The study will involve testing of FFPE (Formalin-fixed paraffin-embedded) blocks with more than 10% tumor content. DNA and RNA will be manually extracted from these samples. The extracted nucleic acids will then be used to prepare libraries using the Oncomine Focus Assay. This assay will simultaneously evaluate 52 genes which comprehensively includes 23 gene fusions, 19 copy number variants, and 35 hotspot genes. After preparation, the libraries will undergo sequencing using Ion Torrent S5 technology. The study is targeted to predict the anticipated side effects to the targeted therapy, assess resistance mechanism in form of presence of clones and subclones and understand clonal and tumor heterogenicity. This approach will allow judicious use of patient tissue and offer cost-effective CGP for Indian NSCLC patients. In addition, the study will improve understanding of tumor genetics for better targeted therapy selection, ultimately improving clinical outcomes.

Key words: NSCLC, FFPE, Molecular Biomarkers, PD-L1, PFS, OS

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1. INTRODUCTION

The lungs are paired organs consisting of the right lung and left lung. Each lung is split into sections called lobes. The right lung has three sections: upper, middle, and lower. The left lung has two sections: upper and lower. Lung is microscopically composed of alveoli. Malignant changes in these alveoli lead to lung carcinoma. Lung cancers rank third and are the leading cause of cancer-related deaths in India, following breast and cervical cancer. Everyday approximately 350 lung cancer patients die which is almost 2.5x of the rate of colorectal carcinoma patients. Globally, approximately 19.3 million new cancer cases were estimated in 2020. India has a significant burden of cancer cases, with an estimated 1.5 million new cases each year (Siegel et al., 2023) (Jani et al., 2022; Jong et al., 2023).

Lung cancers are histopathologically categorized as Non Small Cell Lung Carcinoma (NSCLC) and Small Cell Lung Carcinoma (SCLC). NSCLC is the more common type and comprises 85% of the total number of lung carcinoma cases. NSCLC is further subcategorized as Lung Adenocarcinoma (LUAD), Squamous cell carcinoma (SCC), Large cell lung Carcinoma, Combinations of histological type: Adenosquamous carcinoma, atypical carcinoid, Bronchial gland carcinoma and Sarcomatous carcinoma. The main etiology for NSLCLC is associated with smoking (cigarettes, bidi) along with environmental conditions (air pollutants and occupational hazards). Regions with high smoking rates and environmental pollutants are Asia followed by North America and Europe.

Despite advancements in diagnostics and treatment modalities, survival rates remain dishearteningly low. A 2022 clinical study underscored the urgent need for intensified efforts in improvising clinical outcomes, progression free survival and overall survival of NSCLC patients. Each NSCLC comprises of distinctive clinical features, histomorphology, immunoprofiling and molecular biomarkers- Diagnostics, Predictive, Prognostic And Emerging (Šutić et al., 2021). Due to the identification of biomarkers, there are consistent developments in precision oncology with chemotherapies, targeted therapies, immunotherapies and combination therapies. Mutations in the epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK) and ROS1 genes were the first three clinically actionable biomarkers (George et al., 2024; Shen et al., 2020). However, over the past twenty years, there has been a significant evolution in the lung cancer categories.

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Numerous molecular oncogenic subgroups of non-small cell lung cancer (NSCLC), especially lung adenocarcinoma, have been identified, which alter the prognosis and course of treatment. Recent clinical trial outcomes suggest that newly developed treatments may target specific subsets of lung cancer (Bai et al., 2020).

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2. LITERATURE REVIEW AND RESEARCH GAPS

NSCLC is a heterogenous malignant disease with a high mortality rate and poor prognosis. Approximately 60% of the NSCLC comprises of driver mutations or fusions or combinations of both (SNVs, Insertion, Deletions, Splice Site Variant, CNVs, Fusions). The American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO), and the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) have advocated matching targeted therapies to oncogenic drivers to improve survival and quality of life in oncology practice. Clinical guidelines recommend broad genomic testing methods such as NGS-based panel testing as the findings availed have helped grow the availability of approved and emerging targeted therapies. Comprehensive and broad molecular profiling facilitates the investigation of pertinent co-alterations, such as immune checkpoint inhibitor resistance mutations linked to resistance to targeted therapies (Malapelle et al., 2024; Tan & Tan, 2022) (Padinharayil et al., 2023).

Tobacco smoking is the primary cause of NSCLC. It's crucial to understand how tobacco disrupts cellular signaling mechanisms. This dysregulation promotes changes that can lead to tumor formation. Lung cancers influenced heavily by tobacco primarily result from accumulating genetic changes and evasion of immune surveillance. (Prevention (US) et al., 2010) (Hecht, 1999). Figure 01 describes the impact of tobacco smoking on lung alveoli (cells) and its mechanism which disrupts the normal cellular functioning.

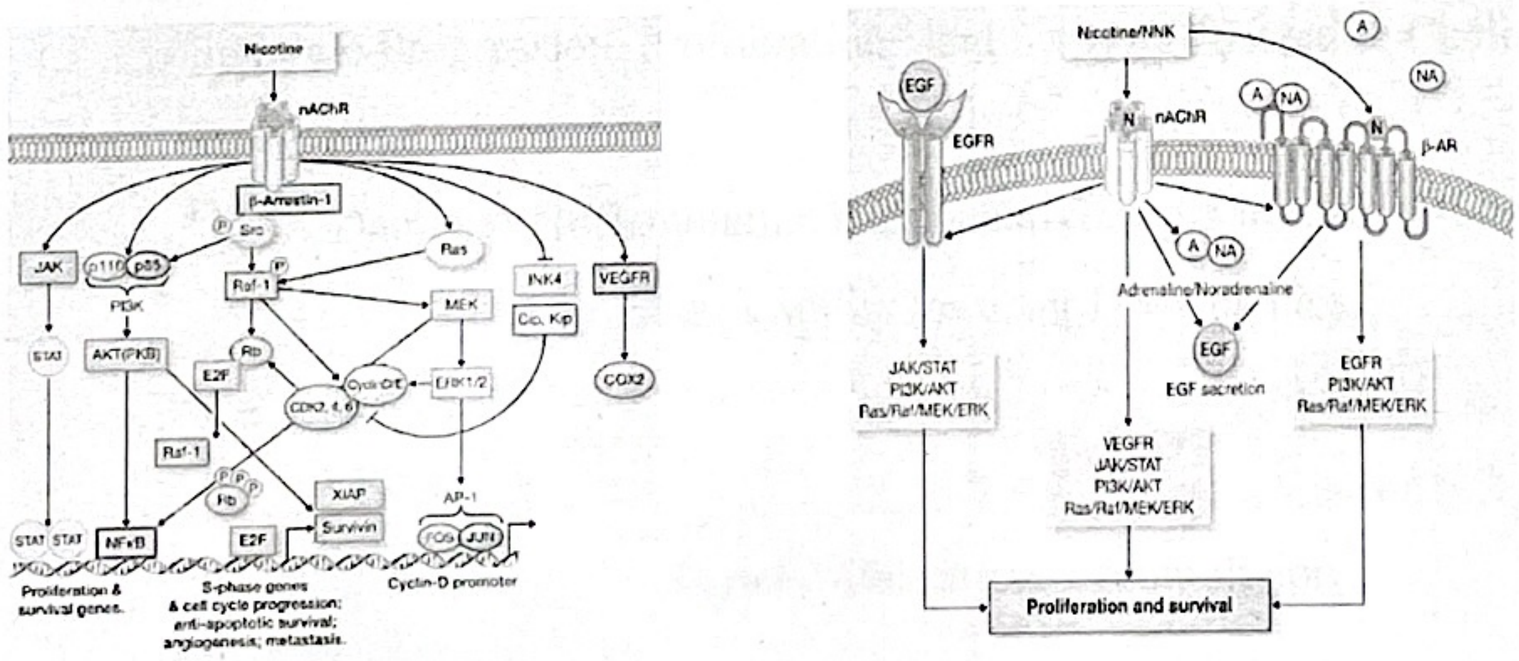

Figure 01: Pathways affected with intake of tobacco, cellular mechanisms leading to NSCLC (Hecht, 1999).

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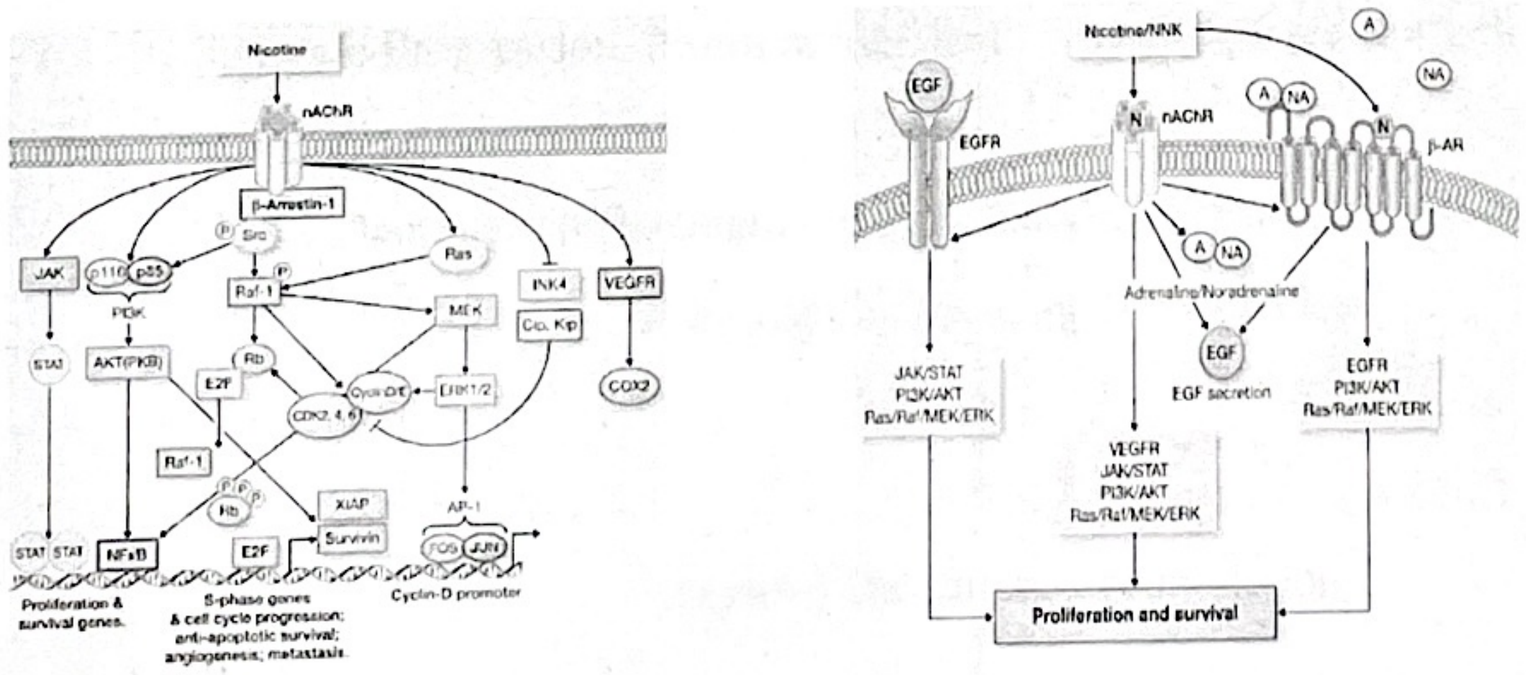

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Polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aromatic amines are well known carcinogens. Nicotine, 4-(methyltyramine)-1-(3-pyridyl)-1-butanone (NNK)

N-nitrosonornicotine (NNN)

↓ Activates

Receptors (Ligand Binding): nicotinic acetylcholine receptors (nAChR), Beta-adrenergic receptors (b-AR)

↓ Activates

Multiple cellular signaling cascades

b-arrestin-1 and Src kinase is recruited to Raf-1 and PI3K/AKT cellular signaling pathways

further cascades to JAK/STAT and Ras/Raf/MAPK pathway

↓

Reduces the levels for-cell cycle inhibitors (p16INK4 and Cip/Kip proteins)

↓ Tumor Growth

Mutagenic effect

Tumor suppressor gene inactivation and oncogene activation

(Cycle Progression, Proliferation, And Survival)(Schaal & Chellappan, 2014)

↓

DNA Damage + Inflammation and oxidative stress in alveoli (interfere with the body's ability to repair DNA damage)

↓

Genetic aberrations accumulation

↓

NSCLC (Sarlak et al., 2020; Xue et al., 2014).

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Based on anatomy and function, lung is divided into

Compartment 1	**Compartment 2**
Parenchyma	Bronchial tree
Function: the apparatus is responsible for respiratory exchange	Function: the apparatus is responsible for delivering air to the alveolar spaces.
Both the compartment are composed of Alveolar cells: Type I : Epithelial component Type II : ciliated columnar cells (associated with higher clearance capacity for the inhaled carcinogen, these are larger particles being trapped in larger bronchioles, secretory cells, goblet cells and basal cells	
While lung tumors under the heavy influence of tobacco smoking are primarily caused by accumulating genetic changes and evading immune monitoring, oncogene-addicted adenocarcinomas are propelled by a single oncogenic mutation with potent transformation activity(Gosney et al., 2023).	

Promptly initiating first-line treatment is crucial for patients with advanced NSCLC. Without treatment, their condition can quickly worsen, potentially making them ineligible for therapy or resulting in death before treatment begins. Accelerating biomarker testing can greatly reduce the risk of these outcomes and help avoid the extra costs linked to delayed care. For instance, the ongoing data from the ADAURA trial, which demonstrates the applicability of adjuvant therapy even for stage 1b disease, highlights the importance of identifying EGFR mutations as early as possible. This should be done in the resected tumor or, preferably, in presurgical specimens if available(Qu et al., 2020; Tan & Tan, 2022).

Additionally, targeted therapies are now becoming established in the neoadjuvant setting. Moreover, optimizing tissue management and ensuring successful analysis of the initial diagnostic specimen will significantly reduce the need for a second biopsy. Any potential financial disadvantages must be weighed against the previously mentioned benefits and considered within the overall cost of managing a lung cancer patient(Eccles et al., 2013). For a patient with a tumor that has a genomic driver or high PD-L1 expression, which makes it

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responsive to immunotherapy, treatment can extend life by many months or even years, resulting in substantial therapy costs over time. As more targets are discovered and more effective drugs are developed, survival rates will continue to improve. (Eccles et al., 2013). Molecular testing is crucial for evaluating these targets, along with PD-L1, to determine eligibility for targeted therapy, immunotherapy, or combination therapies. Identifying mutations that render immunotherapy ineffective allows for more effective treatment options, saving patients from unnecessary and ineffective drugs. This approach not only preserves financial resources but also directs them toward more beneficial treatment options promptly.

The cost of conducting a range of predictive biomarker tests, particularly those based on next-generation sequencing (NGS), is already minimal in comparison to the overall cost of treatment and will continue to decrease in the future. NGS is considered less time-consuming and more cost-effective than single-gene testing when multiple genomic alterations are being investigated, as is increasingly the case with NSCLC(Desai et al., 2021).

Same phenotypes have different genotypes. Similarly, different tumor type with same histology has different driver mutations or fusions. This is known as Intertumor heterogeneity. This occurs due to up or down regulation of particular pathways or combinations of both affecting different pathways(Lovly et al., 2016).

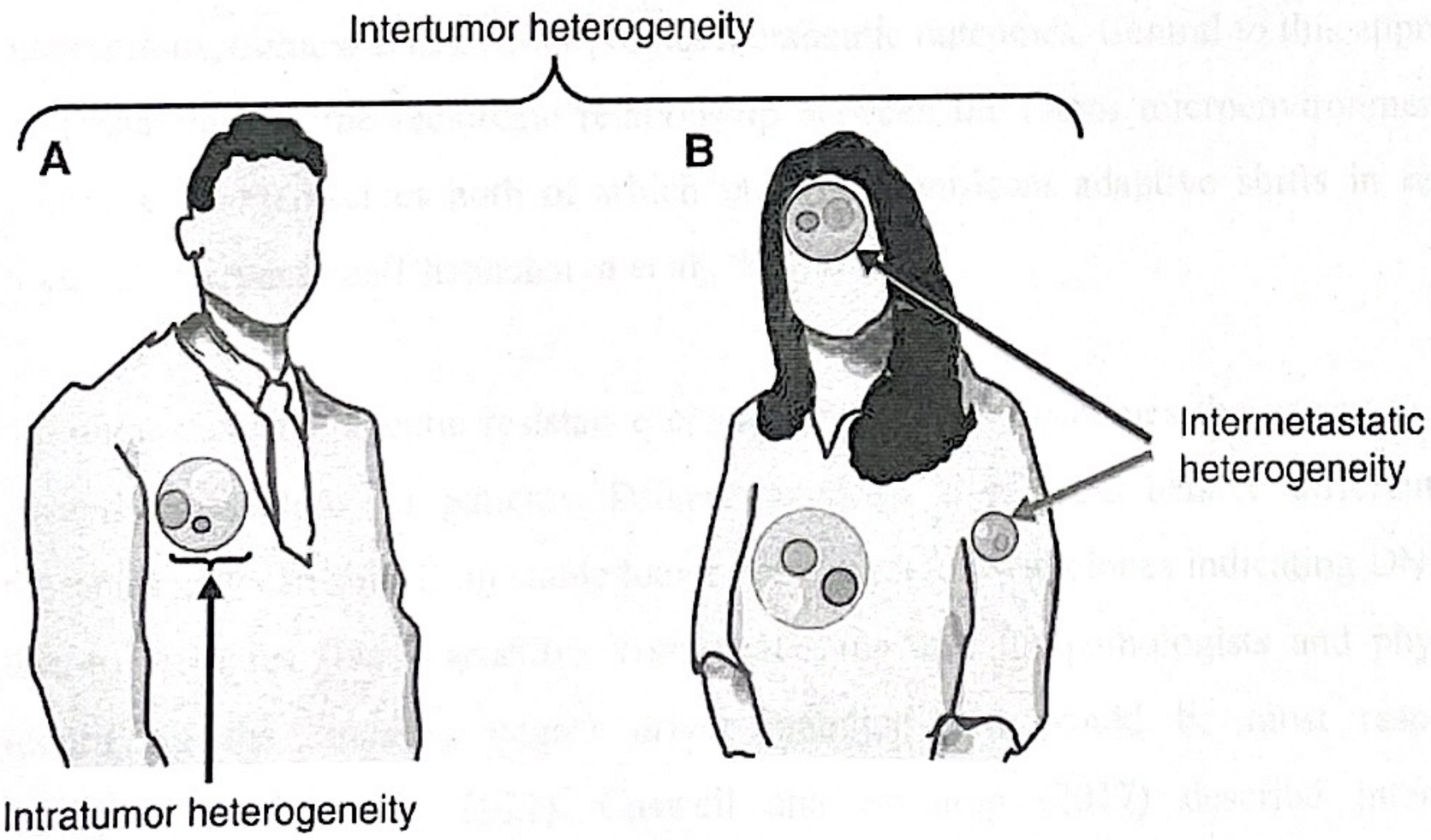

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Figure 02: The above said diagram depict two patient labelled as Patient A and Patient B which explains the intratumor and intertumoral heterogeneity in them respectively. Both patients indicated have lung adenocarcinoma. Intratumor heterogeneity is defined molecular subpopulations within the same tumor type (Patient A) while intertumoral heterogeneity is defined molecularly with subpopulations at different metastatic sites (Patient B) (Senosain & Massion, 2020).

Metrics such as classic pathological traits based on morphologic appearance, invasion and metastasis potential, tumor microenvironment (TME), including the immunological TME (ITME), proliferative index, and growth patterns represent behaviors that can vary significantly. Understanding why patients with the same histologic tumor type may respond differently to identical treatments hinges on identifying critical differences in intertumor heterogeneity. These substantial variations highlight the urgent need to explore the spectrum of molecular subtypes within tumor types(Quail & Joyce, 2013).

Clonal Evolution: Clonal mutations typically represent the foundational events of tumor development preceding the later emergency of subclones mutations. To navigate this complexity, researchers apply game therapy as a theoretical framework to model the evolutionary dynamics and shifting subpopulations that drive intratumor heterogeneity. By analyzing how different treatment sequences, dosages and intervals impact these cellular interactions, clinicians can better predict therapeutic outcomes. Central to this approach is an understanding of the reciprocal relationship between the tumor microenvironment and the cancer cells themselves both of which undergo significant adaptive shifts in response to medical interventions(Bhattacharya et al., 2021).

To understand therapeutic resistance clone, repeat biopsy becomes the necessity. This is a invasive procedure for patients. Driver mutations in NSCLC behave differently during therapies they can shift from stable tumor population to vivid clones indicating DNA damage due to therapies. This variability complicates the task for pathologists and physicians in identifying the "moving target" driver mutation that would be most responsive to treatment(Wölfl et al., 2022). Caswell and Swanton (2017) describe intermetastatic

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heterogeneity as the total heterogeneity of clones and subclones within all metastatic lesions originating from the initial primary lung cancer. Intrametastatic heterogeneity refers to the diversity within a single metastasis. Clinically, it is essential to determine the presence of therapy-resistant clones or subclones within metastatic sites when deciding whether to continue a particular treatment or switch to a new one. Therefore, the optimal type and sequence of therapies for lung cancer should ideally be based on the overall heterogeneity of the primary lung tumor and its metastases(Caswell & Swanton, 2017; Rajadurai et al., 2023).

When there are no identifiable molecular drivers to suggest targeted therapy, immune checkpoint inhibition (ICI) often becomes the most beneficial treatment option. For instance, the KEYNOTE 024 and 042 trials recommend the first-line use of pembrolizumab in advanced non-small cell lung cancer (NSCLC) patients with PD-L1 expression levels of at least 50%, but only in the absence of EGFR and ALK alterations. Combining ICI with other complementary treatments such as chemotherapy, radiotherapy, or targeted agents like EGFR TKIs, VEGFR TKIs, or HDAC inhibitors, as well as using dual agents for ICI like concurrent PD-1/PD-L1 and CTLA-4 inhibition, is an emerging therapeutic strategy for lung cancer. Research across various cancers has demonstrated the potential therapeutic benefits of these ICI combination approaches(Hendriks et al., 2023).

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Role of PDL1 in NSCLC: Monoclonal antibodies that target inhibitory immune checkpoints, like PD-1/PD-L1 and CTLA-4 have been effective in treating several types of solid organ malignancies. Recently, drugs like permbrolizumab, atezolizumab and nivolumab has been approved by FDA as the first line in NSCLC therapeutic choice when PD-L1 is high. Also, recent studies have shown combination therapies like chemotherapy with immunotherapy or targeted therapy with immunotherapy give better clinical outcomes with improvised overall survival irrespective its squamous cell carcinoma or adenocarcinoma. However, one burning issue comes where Pembrolizumab does not work with NSCLC who have high PD-L1 expression highlighting a backend resistance mechanism and opening avenues for broad and comprehensive molecular testing by NGS (Shiravand et al., 2022; Sun et al., 2023; Wojtukiewicz et al., 2021).

PD-L1 is a known ligand on programmed death receptor (PD-1) receptor and is a transmembrane protein present on T cells. Recent findings suggest that PD-L1 also promotes tumor cell survival, cancer growth, dissemination, and resistance to therapies, although the specific intracellular signaling pathways induced by PD-L1 are still not well understood. PD-L1 expression can be started by signals from outside the cell, like interferon-gamma, or by signals inside the cell, such as when the mammalian target of rapamycin (mTOR) or mitogen-activated protein kinase (MAPK) pathways are activated. However, the primary factors influencing its baseline expression remain unclear. Tobacco smoking is notably linked to T cell exhaustion and PD-1 upregulation, which contribute to immune evasion. Lung cancer in smokers often shows a high load of neoantigens, increased immunogenicity, and elevated PD-L1 expression. This is important because research has shown that people with advanced non-small cell lung cancer who still smoke and have positive PD-L1 levels are more likely to benefit from treatment with anti-PD-1 drugs than those who have never smoked (Han et al., 2020).

Mutations in NSCLC are categorized as Oncogenes and Tumor suppressor genes. Oncogene are Proto-oncogenes. They usually help cells grow and divide so they can make more or so they can live longer. A proto-oncogene becomes activated and loses its ability to function when it mutates or when there are too many copies of it. This may cause the cell to begin

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growing uncontrollably, which could result in cancer. Tumor suppressor genes are normal genes that either stop cells from proliferating or give instructions to die when the time comes; this process is known as apoptosis, or programmed cell death. Tumor suppressor gene malfunctions can cause cells to proliferate uncontrollably, which can result in cancer. (Kontomanolis et al., 2020).

Non-small cell lung cancer (NSCLC), exhibits aggressive biological behavior, swiftly advancing from early to advanced stages, which limits opportunities for timely and apt therapeutic intervention. The disease undergoes clonal evolution. Its genetic and molecular complexity renders it highly heterogeneous, complicating treatment approaches and fostering resistance mechanisms. Developing country like India, Medical oncologist utilize molecular testing for choosing therapeutic regimens. They are broadly characterized into RT PCR Panels (Targeted approach), Sanger Sequencing (Gold Standard), Immunohistochemistry (Protein Exressions) and FISH (Fluorescence in situ Hybridization). For cost benefits to the patient, most common ALK and ROS1 testing by IHC or FISH is opted by the clinician and EGFR by RT PR making it quick and cost effective in view of most common mutated drivers. In case if all these testing is not detected upfront Next generation sequencing (NGS) is opted with short panels and most common driver mutations with 12 or 16 genes. The most common loss happens is tissue as firstly due to IHC OR FISH OR RT PCR testing where the results are not detected there is loss of tissue and tumor where the precious tissue and subclone of tumors are lost leading to more chances of failures in view of tissue depletion, insufficient or low tumor content block testing and failures at bioinformatics tool.

It's essential to assess molecular subtypes in advanced and metastatic NSCLC is vital for predicting therapeutic outcomes and choosing better treatment opportunities in the era of precision oncology, as it helps to understand mutational landscape in form of co-occurrence: short nucleotide variants, copy number gains, fusions, insertions or deletions. as it uncovers the specific resistant clones and subclones within a patient's unique tumor profile. This helps in tumor profiling. This further can be investigated for PD-L1 for the eligibility of immunotherapy in comparison to standard of care traditional chemotherapy, radiation, combination therapy. This will help to bridge the gap between genomics and proteomics and

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help integrating treatment strategies and resolving drug toxicities and wrong treatment to the patient. Time is everything in a cancer patient.

Chemotherapy practically becomes the first line of treatment in such cases. Nonetheless, chemotherapy regimens, platinum-based (e.g., cisplatin, carboplatin) or non-platinum agents (e.g., pemetrexed, gemcitabine, docetaxel) are infamous due to their debilitating side effects. Nausea, vomiting, neurotoxicity, myelosuppression, and other side effects are common and often affect the quality of life of patients to a great extent. These serious side effects often require dose changes or breaks in treatment that subsequently can reduce the overall efficacy of chemotherapy and result in worse treatment results. This also affects the health and finances of the patients.

IDENTIFIED GAPS IN LITERATURE:

Non-small cell lung cancer (NSCLC) is a highly aggressive malignancy characterized by a low overall survival rate of 7.5-10%. Despite extensive research efforts, therapeutic responses remain challenging. This results in poor clinical outcomes, limited progression-free survival, patient mortality and significant financial burden due to the high costs of treatment. To address this, molecular testing offers a superior approach by elucidating the underlying causes of cancer. This approach helps in deciphering a particular patient tumor molecular composition, and guiding clinicians in targeting these molecular characteristics. This approach is significantly more cost-effective compared to the high expenses associated with non-targeted therapies.

Further research is required to optimize the use of targeted therapies in the combination therapies in advanced NSCLC. This involves evaluating the effectiveness and practicality of performing comprehensive molecular testing on initial diagnostic samples, encompassing multiple genes testing. The further need for repeat biopsies is also eliminated due to tumor depletion in single gene testing. There is a scope in more informed cases.

It is essential to understand the molecular subtypes in advanced and metastatic settings for predicting treatment response and developing personalized therapy options. This approach also helps in identifying therapy resistance clones and subclones which are present in a

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particular patient tumor. Investigating the efficacy and safety of combining immune checkpoint inhibitors (ICIs) with other modalities like chemotherapy, radiotherapy, or targeted agents is essential for advancing treatment strategies in NSCLC. There is a need to establish improved access to therapeutic opportunities provided by comprehensive genomic profiling and precision medicine approaches, ensuring these are effectively integrated into clinical practice. Addressing these gaps through further research will contribute to improving outcomes and reducing the overall burden of NSCLC on patients and healthcare systems globally.

The research question arises can comprehensive genomic profiling (CGP) using Next Generation Sequencing (NGS) be implemented in Indian NSCLC patients to better understand the mutation landscape and identify emerging biomarkers that could improve clinical outcomes and progression-free survival. We hypothesis since the mutational landscape of the Indian population remains less studied, we anticipate that conducting this study will provide a distinct mutational profile. This will assist Indian clinicians in understanding the genetic composition of tumors more effectively.

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3. DESCRIPTION OF THE BROAD AREA AND PROBLEM STATEMENT

The general field is the research on non-small cell lung cancer (NSCLC) in terms of designing therapeutic approaches, especially the role of immune checkpoint inhibitors (ICIs), targeted therapy and tumor heterogeneity knowledge. Although there is progress in the treatment of NSCLC, there are still considerable challenges to overcome since tumor heterogeneity and dynamic changes of cancer mutations make it difficult to treat cancer. The following are some pertinent statistics data to demonstrate the size of the issue:

Incidence and Mortality: Lung cancer has been the major cause of deaths due to cancer all over the world. The number of new cases of lung cancer and deaths related to this disease is estimated to be 2.2 million and 1.8 million worldwide in 2020, respectively. In the United States, it is estimated that in 2023, there will be about 238,340 new cases of lung cancer and 127,070 deaths from the disease. Survival Rates: 5-year survival of NSCLC differs greatly according to the stage of diagnosis. In localized NSCLC (one that is limited to the lungs), the survival rate is approximately 63% after 5 years. But when the cancer has moved to regional lymph nodes the rate is only 35% and when the cancer has moved far away it is only about 7%. Altogether, the survival of NSCLC in 5 years is about 25%. Dynamic Nature of Mutations: The development of resistance mutations and tumor evolution has been a major problem in the treatment of NSCLC. As an example, approximately 50-60 percent of patients that respond to initial targeted therapy using EGFR-mutant NSCLC will develop resistance as a result of the T790M mutation. These data demonstrate the multifacetedness and seriousness of NSCLC, and the necessity of new and dynamic approaches to treatment to overcome tumor heterogeneity and mutational changes. The research and discovery of the various molecular pathways and resistance mechanisms is vital in the quest to establish more individualized and efficient therapeutic approaches.

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4. RESEARCH OBJECTIVES

1. To determine the prevalence of diagnostic, predictive, prognostics and emerging molecular markers of Non-Small Cell Lung Carcinoma in Indian Cohort.
2. To identify variants of Non-Small Cell Lung Carcinoma in an Indian cohort and map them to actionable variants in an Indian patient cohort and map them to cellular pathways.
3. To correlate the mutational landscape of Non-Small Cell Lung Carcinoma with PD-L1 expression by immunohistochemistry in different tumor grades.

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5. METHODOLOGY TO BE ADOPTED/RESEARCH METHODOLOGY

Step 1: Patient Recruitment

One hundred and twenty (120) patients with an established diagnosis of non-small cell lung carcinoma (NSCLC) (both early and advanced stages) will be recruited into the study. All the participating patients will be informed about their participation in order to comply with ethics.

Step 2: Sample Collection

Each patient will have formalin-fixed paraffin-embedded (FFPE) blocks that will store tumor tissue that can be used in molecular testing. These blocks will be the main material of further analyses.

Step 3: Sample Screening

for Inclusion

a) Tissue Sectioning and staining: The FFPE blocks collected will be sliced into 5-micrometer-thick slices. These slices will be stained using hematoxylin and eosin (H&E) to enable the use of histological analysis.

b) Tumor Content Screening: H & E stained slides will be examined by a Molecular Pathologist to determine tumor content. Samples will be put under molecular testing provided that they satisfied the following criteria: Tumor content of more than 10%. Less than 20% necrotic.

• Histopathological Grading: The H&E-stained slides will be further assessed to be histopathologically graded with NSCLC. On the basis of differentiation, there are going to be three groups of samples: Well Differentiated Moderately Differentiated Poorly Differentiated

Step 4: Sample Processing

After the specimens (FFPE blocks) have passed the inclusion criteria, the following procedures will be carried out on them: a) Nucleic Acid Extraction and Quantification: Nucleic Acid Extraction: DNA and RNA: To guarantee quality nucleic acids that can be used

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in downstream applications, DNA and RNA will be manually removed out of the FFPE blocks using CE-IVD certified protocols. DNA and RNA quantification:

The samples were quantified using the qPCR kit. The RNA and DNA extracted will be measured with the help of Qubit and Nanodrop tools, which will help measure the concentration and quality. Sample selection: Only those samples that are going to meet the necessary quality and quantity requirements will be processed further.

Library Preparation: Qualified DNA and RNA samples will be prepared by Oncomine Focus Assay that will be used in preparation of libraries that will target a broad set of oncogenes and tumor suppressor genes applicable in the case of NSCLC.

TapeStation profiles will be created to determine the size distribution and integrity of the prepared libraries.

Sequencing: The sequencing of the prepared libraries will be performed with the Ion Torrent S5 platform that will offer better high-throughput sequencing of the genomic landscape of the sample.

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Gene List: Oncomine Focus Assay

Hotspot genes (35 genes)		Copy number genes (19 genes)		Gene fusions (23 genes)	
AKT1	*IDH2*	*AKT1*	*PIK3CA*	*ABL1*	*PDGFRA*
ALK	*JAK1*	*ALK*	*PDGFRA*	*AKT3*	*PPARG*
AR	*JAK2*	*AR*		*ALK*	*RAF1*
BRAF	*JAK3*	*BRAF*		*AXL*	*RET*
CDK4	*KIT*	*CCND1*		*BRAF*	*ROS1*
CTNNB1	*KRAS*	*CDK4*		*EGFR*	
DDR2	*MAP2K1*	*CDK6*		*ERBB2*	
EGFR	*MAP2K2*	*EGFR*		*ERG*	
ERBB2	*MET*	*ERBB2*		*ETV1*	
ERBB3	*MTOR*	*FGFR1*		*ETV4*	
ERBB4	*NRAS*	*FGFR2*		*ETV5*	
ESR1	*PDGFRA*	*FGFR3*		*FGFR1*	
FGFR2	*PIK3CA*	*FGFR4*		*FGFR2*	
FGFR3	*RAF1*	*KIT*		*FGFR3*	
GNA11	*RET*	*KRAS*		*MET*	
GNAQ	*ROS1*	*MET*		*NTRK1*	
HRAS	*SMO*	*MYC*		*NTRK2*	
IDH1		*MYCN*		*NTRK3*	

b) Immunohistochemistry (IHC) Testing:

PD-L1 Testing:

Manual immunohistochemistry (IHC) testing for PD-L1 expression will be conducted on the FFPE tissue sections.

This step involved staining the tissue sections with PD-L1 antibodies and will evaluate the expression levels further determining their potential role in therapy response and prognosis.

Step 5: Data Analysis

a) Bioinformatic Analysis of Sequencing Data:

Quality Control:

- After sequencing, bioinformatic quality control (QC) files will be generated on the Ion Torrent server.
- Samples that passed QC will be further processed.
- Conversion to Variant calling files (VCFs)

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- The sequenced data will be converted into VCF files and will be uploaded to the Ion Torrent Reporter for analysis.

VCF Analysis:

- The VCFs will be analyzed using DNA and RNA workflows available on the Ion Torrent Reporter.
- Results from these analyses will be tabulated for further evaluation.

Annotation and Variant Analysis:

- Variant annotation will be performed using Integrated Genome Viewer (IGV) to ensure accurate identification and categorization.
- Variants will be categorized into:

✓ DNA: Single Nucleotide Variants (SNVs), Insertions, Deletions, Splice Site Variants, Copy Number Variants (CNVs)

✓ RNA: Fusions

Classification of variants:

- Variants will be classified according to guidelines from the American College of Medical Genetics (ACMG), Association for Molecular Pathology (AMP), and College of American Pathologists (CAP) into:
- Oncogenic more commonly attributed to Pathogenic
- Likely Oncogenic more commonly attributed to Likely Pathogenic
- Variant of Uncertain Significance (VUS)
- Benign and likely benign variants were not considered for further analysis.

Tiering and Pathway Analysis:

- Variants will be further classified based on tiering, considering drug availability and variant effect.
- Affected pathways will be recorded to understand their roles in tumorigenesis and therapeutic resistance.

b) Immunohistochemistry (IHC) Analysis:

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- PD-L1 Expression Analysis:
- The results from IHC testing for PD-L1 expression will be analyzed.
- The extent of PD-L1 expression on cancerous cells will be captured to be associated with therapeutic effects and prognosis of patients.

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6. EXPECTED OUTCOME OF THE RESEARCH

Application of next-generation sequencing (NGS) to non-smallcell lung carcinoma (NSCLC) research and clinical practice will bring positive results especially in targeted therapeutics which is essential in enhancing clinical outcomes and patient survival. Through offering a range of molecular profiling, NGS will allow the identification of practical genetic changes that will guide the choice of targeted therapies, resulting in more efficient treatment and improved clinical outcomes of NSCLC patients. The important molecular changes, as well as the less frequent mutations that can act as the emerging biomarkers of the treatment response, enable the personalized treatment decision-making. Moreover, NGS offers useful information about tumor heterogeneity and clonal evolution that can be used to improve our knowledge of tumor progression and resistance to treatment in NSCLC. Although NGS-based comprehensive profiling interpretation and standardization might be difficult, its integration into clinical practice has immense potential to streamline the management of NSCLC and enhance patient care. Going forward, further studies are required to perfect NGS methods further, find new targets of therapy, and overcome resistance in NSCLC. With the power of NGS, the entire potential of precision medicine can be realized, which ultimately will benefit the patients of NSCLC. The work will also shed light on the contribution of Precision oncology to NSCLC. Precision Oncology is a game changer in the treatment of non-small cell lung carcinoma (NSCLC) by developing the individualized approach to treatment based on the genetic and molecular profile of their tumor. The most important features of the role of precision oncology in the treatment of NSCLC are as follows: Comprehensive Molecular Profiling: Precision oncology entails the detailed molecular profiling of tumors with cutting-edge technologies like next-generation sequencing (NGS). It is possible to identify certain genetic mutations, alterations, and biomarkers that promote tumor growth and progression in NSCLC. Selection of Targeted Therapy: Precision oncology allows the selection of targeted therapies, which selectively suppress the activity of known genetic changes, based on the molecular profile. It will result in more efficient and individualized treatment plans and enhance clinical outcomes. Enhanced Therapeutic Response: Precision oncology increases the chances of success of the treatment process

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and reduces exposure to unproductive therapies by pairing patients with the most likely to be effective therapeutic options based on the tumor genetic composition. Conquering Therapeutic Resistance: Precision oncology aids in comprehending how tumor therapeutic resistance happens by examining how tumors have evolved over time, genetically. This knowledge can inform the change of treatment approaches to overcome resistance and ensure therapeutic efficacy. Prognostic and Predictive Biomarkers: Molecular profiling is used to identify both prognostic and predictive biomarkers which help in predicting both the outcome of the disease and responses to treatment. This can be used to stratify risks better and make more informed clinical decisions. Individualized Treatment Plans: Precision oncology is shifting to the one-size-fits-all paradigm to a more personalized treatment paradigm, in which therapies are tailored to the specific tumor characteristics of the individual patient. This results in an improved management of NSCLC and patient quality of life. Clinical Trial Matching: Clinical trials of new targeted therapies can be offered to patients with certain genetic alterations. Precision oncology will assist in aligning patients with appropriate clinical trials, which will provide access to state-of-the-art therapies and help in advancing the research of NSCLC. Less Toxicity: Precision oncology has the potential to minimize toxicity of conventional chemotherapy by targeting a particular molecular change. Specific therapies are usually better tolerated with fewer side effects, which result in better patient compliance and adherence to therapy. Improved Tumor Biology Knowledge: Precision oncology will help to further understand the biological pathways underlying NSCLC. The knowledge is vital in the creation of novel therapeutic measures, as well as the ongoing enhancement of patient care. Overall, precision oncology is a paradigm shift in NSCLC treatment that can use molecular insights to provide highly personal and effective therapies. It increases the effectiveness of treatment, reduces resistance, helps to improve patient outcomes, and preconditions the future of cancer care.

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Mutational Landscape and Actionable Biomarkers In Non-Small Cell Lung Carcinoma: A Next Generation Sequencing Based Study In Indian Cohort

SHORT SYNOPSIS

by

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Under the supervision of

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ABSTRACT

Immunotherapy, either alone or in combination with chemotherapy or targeted therapy, is now the standard of care for advanced Non-Small Cell Lung Cancer (NSCLC). The 5-year progression-free survival rate is low, which is 7.5-10%. Therefore, there is an urgent need to optimize treatment strategies for improvising clinical outcomes. The National Comprehensive Cancer Network (NCCN) has advised molecular testing, which involves using a comprehensive gene panel (CGP) testing. Each phenotype exhibits a distinct genotype. Earlier studies on CGP using NGS have revealed crucial signaling pathways and resistance mechanisms across multiple genes in each patient's tumor. The co-relation with PD-L1 expression has further guided the decision making towards patients' eligibility for Immunotherapy or combination therapies. The mutational landscape and resistance mechanisms of the Indian population remain less studied. Hence, this study aims to understand the mutational landscape, prevalence of resistance mechanisms and correlation of CGP with PD-L1 expression, in Indian population. The study will involve testing of FFPE

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(Formalin-fixed paraffin-embedded) blocks with more than 10% tumor content. DNA and RNA will be manually extracted from these samples. The extracted nucleic acids will then be used to prepare libraries using the Oncomine Focus Assay. This assay will simultaneously evaluate 52 genes which comprehensively includes 23 gene fusions, 19 copy number variants, and 35 hotspot genes. After preparation, the libraries will undergo sequencing using Ion Torrent S5 technology. The study is targeted to predict the anticipated side effects to the targeted therapy, assess resistance mechanism in form of presence of clones and subclones and understand clonal and tumor heterogenicity. This approach will allow judicious use of patient tissue and offer cost-effective CGP for Indian NSCLC patients. In addition, the study will improve understanding of tumor genetics for better targeted therapy selection, ultimately improving clinical outcomes.

Key words: NSCLC, FFPE, Molecular Biomarkers, PD-L1, PFS, OS

1. INTRODUCTION

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